THE INFLUENCE OF NORMAL BEEF SERUM ON THE ANTHRAX BACILLUS

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Our interest in this subject was aroused by the numerous reports of Penna, Cuenca and Kraus[1] on the favorable results in the treatment of the pustule and bacteremia of human anthrax with ordinary beef serum. These investigators injected normal beef serum, previously heated twice at 56 C. for 30 minutes for purposes of sterilization, in doses of 30 to 50 c c repeated in 12, 24 or 36 hours, as the case might require; the injections were made subcutaneously or intramuscularly except in very severe cases when the serum was given intravenously. According to their latest report on 200 cases, the mortality has been reduced to 0.5% as compared with 10% in 250 cases treated in the usual manner during the preceding 10 years. In an experimental study Kraus and his associates found normal beef serum just as effectual as horse anti-anthrax serum in the protection of rabbits against virulent anthrax bacilli.

Solari[2] has reported favorably on the treatment of 6 cases of anthrax with heated normal beef serum, and Langon[3] also has made a favorable report on 13 cases; Lignieres,[4] however, has reported unfavorably on the curative action of normal beef serum, stating that it is inferior to horse anti-anthrax serum and called attention to the prevalence of anthrax in cattle as evidence of the apparent lack of natural resistance to this disease and of defensive properties in their blood.

PURPOSE OF INVESTIGATION

Our studies were made with heated and unheated beef serum collected in abattoirs and under asceptic conditions from ordinary herd cattle; all of the animals were full grown and healthy at the time of

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[1] Prensa Medica Argentina, 1917, 3, 297; 1917, 4, 91 and 147; 1918, 4, 455.
[2] Semana méd., 1917, 24, 98.
[3] Ann d. l. Faculated d. Med., 1918, 3, 258.
[4] Prensa Medica Argentina, 1917, 4, 49, 370.

bleeding, but the histories were unknown in relation to previous attacks of anthrax.

The object was to study the protection and curative properties of beef serum in anthrax infections in rabbits and mice, and also to determine if beef serum contained bactericidins, agglutinins and complement fixing substances for anthrax bacilli.

Inasmuch as rabbits were found highly immune and white mice highly susceptible to the culture of anthrax bacilli employed in this investigation, the serum of these animals was included in the study for the purpose of comparison with beef serum and for study of the mechanism of the nature of natural immunity to anthrax.

TOXICITY OF BEEF SERUM FOR MICE

The majority of our protection tests were made with white mice inasmuch as rabbits were found too highly resistant to anthrax bacilli; accordingly, the toxicity of heated and unheated beef serum for mice was determined by intraperitoneal injection preliminary to the protection tests.

The general result of these tests was to show that fresh, unheated beef serum is but slightly toxic for white mice and that heated serum occasionally is somewhat less toxic. Mice receiving intraperitoneal injections of fresh, sterile beef serum in a dose of 0.2 c c per gram of body weight, equivalent to 5 c c undiluted serum for a 25 gm. mouse, occasionally died within 48 hours after injection, while similar doses of the same serum heated twice at 56 C. for 30 minutes rendered some of the mice toxic for from 24 to 48 hours followed by recovery. Mice receiving 0.1 c c of unheated or heated serum per gram of weight, or 2.5 c c for a 25 gm. mouse and equivalent to 100 c c per kilogram of weight usually survived for an indefinite period.

VIRULENCE OF ANTHRAX BACILLI FOR MICE AND RABBITS

All experiments were made with a single strain of the anthrax bacillus, kindly furnished by Dr. John Reichel, which was found to possess a high degree of virulence readily maintained by occasional passage through mice.

Twenty-four hour broth cultures of this strain thoroughly suspended by shaking with glass beads, regularly killed mice weighing from 14 to 18 gm. in 48 hours in a dose of 0.00002 c c injected intraperitoneally, the bacilli

invariably being recovered from the blood of the heart; half this dose, or 0.00001 c c, killed mice of these weights occasionally within 48 hours, the survivors living at least 2 weeks or longer. In the protection tests with beef serum the minimal lethal dose of culture was taken as 0.00002 c c, equivalent to 0.2 c c of a 1:10,000 dilution or about 0.001 c c per kilogram of weight, and the virulence was readily maintained at this level for a period of several months.

The extremely high resistance of rabbits to the same culture is indicated by the fact that the minimal lethal dose was about 2,000 to 3,000 times greater than for mice, being about 3 c c of undiluted broth culture per kilogram of body weight injected intravenously; this dose usually killed in about 48 hours, 2 c c per kilogram in about 96 hours, while 1 c c per kilogram was usually without fatal effects over an indefinite period of time. Among the rabbits succumbing to these large doses within 96 hours after infection, anthrax bacilli were only occasionally recoverable from the blood of the heart.

TABLE 1

THE PROTECTIVE VALUE OF STERILE UNHEATED NORMAL BEEF SERUM FOR MICE INFECTED WITH B. ANTHRACIS

Weight in Grams	Administration of Serum	Duration of Life in Hours after Infection
26	12 hours before bacilli	72
24	12 hours before bacilli	44
25	6 hours before bacilli	28
21	6 hours before bacilli	26
21	2 hours before bacilli	122
23	2 hours before bacilli	28
26	Simultaneously with bacilli	36
29	Simultaneously with bacilli	60
24	2 hours after bacilli	36
26	2 hours after bacilli	Lived indefinitely
21	Culture control	48
23	Culture control	46
20	Serum control	Lived indefinitely

Mice infected by intraperitoneal injection of 2 M. L. D. (= 0.00004 c c culture).
Each mouse received 1 c c serum per 100 gm. of weight intraperitoneally.

Table 1 shows the results of an intraperitoneal injection of mice with 1 c c of fresh, unheated beef serum per 100 gm. of weight, equivalent to 10 c c per kilogram, at varying intervals before and after the intraperitoneal injection of 0.00004 c c of a broth culture of B. anthracis, equivalent to two minimal lethal doses; the culture controls died at the close of the second day after infection, and while the lives of two mice receiving serum were prolonged beyond this period, the results were irregular and failed to show protective or curative properties on the part of this serum. Anthrax bacilli were recovered from the blood of the heart of each dead mouse; the serum control lived indefinitely.

Table 2 shows the results of a similar experiment with a larger dose of serum, namely, 10 c c per 100 gm. of weight, equivalent to 100 c c per kilogram; each mouse was infected with two minimal lethal doses of culture, the culture controls succumbing in about 48 hours. The serum control lived indefinitely, and the general results of the experiment were that this serum failed appreciably to protect the mice.

TABLE 2

THE PROTECTIVE VALUE OF STERILE UNHEATED NORMAL BEEF SERUM WITH B. ANTHRACIS

Weight in Grams	Administration of Serum	Duration of Life in Hours after Infection
14	4 hours before bacilli	Lived indefinitely
18	4 hours before bacilli	34
22	2 hours before bacilli	40
18	2 hours before bacilli	24
22	Simultaneously with bacilli	36
18	Simultaneously with bacilli	24
14	2 hours after bacilli	36
27	2 hours after bacilli	34
26	4 hours after bacilli	24
16	4 hours after bacilli	36
18	6 hours after bacilli	Lived indefinitely
16	6 hours after bacilli	48
27	Culture control	48
16	Serum control	Lived indefinitely

Each mouse was infected by intraperitoneal injection of 2 M. L. D. of culture (= 0.00004 c c culture).
Each mouse received 10 c c serum per 100 gm. of weight intraperitoneally.

TABLE 3

THE PROTECTIVE VALUE OF STERILE UNHEATED NORMAL BEEF SERUM FOR MICE INFECTED WITH B. ANTHRACIS

Weight in Grams	Administration of Serum	Duration of Life in Hours after Infection
12	4 hours before bacilli	26
14	4 hours before bacilli	26
11	2 hours before bacilli	23
12	2 hours before bacilli	26
11	Simultaneously with bacilli	26
12	Simultaneously with bacilli	26
14	2 hours after bacilli	24
11	2 hours after bacilli	24
14	4 hours after bacilli	48
13	4 hours after bacilli	48
13	6 hours after bacilli	50
12	6 hours after bacilli	48
13	Culture control	24
14	Culture control	24
15	Serum control	Lived indefinitely

Each mouse was infected by intraperitoneal injection of 5 M. L. D. of culture (= 0.0001 c c).
Each mouse received 2 c c serum per 100 gm. of weight intraperitoneally.

Table 3 shows the results of the administration of 2 c c of fresh, unheated beef serum per 100 gm. of weight, equivalent to 20 c c per kilogram, at intervals before and after the injection of 5 minimal lethal doses of culture. As expected, the culture controls succumbed more quickly with the heavier infection; while the lives of 4 mice were prolonged 24 hours beyond the controls, the general results bearing on the protective value of the serum were negative.

Additional experiments were made with repeated injections of one minimal lethal dose of culture at intervals of 24 hours followed one hour after each injection of culture by intraperitoneal injection of 1 c c of fresh, unheated beef serum per 100 gm. of weight, equivalent to 10 c c per kilogram; the

results of an experiment are shown in table 4 and indicate a lack of appreciable protective value on the part of normal beef serum.

TABLE 4

THE PROTECTIVE VALUE OF REPEATED DOSES OF STERILE UNHEATED NORMAL BEEF SERUM FOR MICE RECEIVING REPEATED INJECTIONS OF B. ANTHRACIS

Weight in Grams	Number of Injections of Culture	Number of Injections of Serum 2 Hours after Culture	Duration of Life in Hours
23	2	2	52
25	2	2	44
19	2	2	36
27	3	3	72
23	2	None	48
18	2	None	47
23	None	2	Lived indefinitely

By intraperitoneal injection; 1 M. L. D. (= 0.00002 c c culture) every 24 hours.
By intraperitoneal injection; 1 c c serum per 100 gm. of weight every 24 hours administered 1 hour after the culture.

TABLE 5

THE PROTECTIVE VALUE OF UNHEATED AND HEATED BEEF RABBIT SERUMS FOR MICE INFECTED WITH INCREASING AMOUNTS OF B. ANTHRACIS

Weight in Grams	Culture	Serum	Duration of Life in Hours
20	12 M. L. D. (control)	None	48
22	6 M. L. D. (control)	None	24
24	3 M. L. D. (control)	None	48
17	12 M. L. D.	Beef No. 1 unheated	48
26	6 M. L. D.	Beef No. 1 unheated	24
21	3 M. L. D	Beef No. 1 unheated	24
17	12 M. L. D.	Beef No. 1 heated	24
14	6 M. L. D.	Beef No. 1 heated	24
17	3 M. L. D.	Beef No. 1 heated	24
14	12 M. L. D.	Beef No. 2 unheated	24
20	6 M. L. D.	Beef No. 2 unheated	48
17	3 M. L. D.	Beef No. 2 unheated	48
16	12 M. L. D.	Beef No. 2 heated	24
17	6 M. L. D.	Beef No. 2 heated	48
16	3 M. L. D.	Beef No. 2 heated	24
19	12 M. L. D.	Rabbit No. 1 unheated	24
16	6 M. L. D.	Rabbit No. 1 unheated	24
17	3 M. L. D.	Rabbit No. 1 unheated	24
15	12 M. L. D.	Rabbit No. 1 heated	24
14	6 M. L. D.	Rabbit No. 1 heated	48
16	3 M. L. D.	Rabbit No. 1 heated	48
18	None	Beef No. 1 unheated	Lived indefinitely
14	None	Beef No. 2 unheated	Lived indefinitely
17	None	Rabbit No. 1 unheated	Lived indefinitely
21	None	Rabbit No. 1 heated	Lived indefinitely

Injected intraperitoneally; 1 M. L. D. equaled 0.00002 c c 24-hour broth culture.
Injected intraperitoneally one hour after culture in dose of 1 c c per 100 gm.

The experiment in table 5 was made by infecting a series of mice with increasing doses of culture equivalent to 3, 6 and 12 minimal lethal doses followed in an hour by the intraperitoneal injection of a dose of unheated and heated normal beef and rabbit serums equivalent to 1 c c per 100 gm., or 10 c c per kilogram. The serums were heated twice at 56 C. for thirty minutes. Rabbit serum was included, by reason of the high resistance of rabbits to the anthrax bacillus, for the purpose of studying the possible protective value of this serum for mice. All of the serum controls lived indefinitely;

the culture controls succumbed in from 24 to 48 hours with anthrax bacteremia. Neither heated nor unheated normal beef or rabbit serum showed appreciable protective properties.

As previously stated, the natural resistance of rabbits to virulent anthrax bacilli was so high and variable as to render our experiments with these animals on the protective and curative properties of normal beef serum very uncertain and difficult of interpretation; the results of one experiment are shown in table 6.

In this experiment rabbits were injected intravenously with 1 c c of undiluted broth culture of B. anthracis per kilogram of weight every 24 hours followed in an hour by the intravenous injection of 2 c c of sterile normal beef serum (unheated) per kilogram of weight. The culture control died after 4 injections; the serum control received 6 injections and died on the eleventh day, or 5 days after the last injection of serum. The rabbits receiving culture and serum died in from 2 to 7 days and failed to show in this and similar experiments an undoubted protective value of normal beef serum for the culture employed.

TABLE 6

THE PROTECTIVE VALUE OF REPEATED DOSES OF STERILE UNHEATED NORMAL BEEF SERUM FOR RABBITS RECEIVING REPEATED INJECTIONS OF B. ANTHRACIS

Weight in Grams	Number of Injections of Culture	Number of Injections of Serum 2 Hours after Culture	Duration of Life
1326	4	4	7 days
1572	2	2	52 hours
1826	4	4	6 days
1246	4	None	6 days
1400	None	6	11 days

By intravenous injection; 1 c c of broth culture per kilogram of weight every 24 hours.
By intravenous innjection; 2 c c of serum per kilogram of weight every 24 hours one hour after the injection of culture.

TABLE 7

THE BACTERICIDAL ACTIVITY OF NORMAL BEEF AND RABBIT SERUMS FOR B. ANTHRACIS

Weight in Grams	Final Dilutions of Serum	Duration of Life in Hours
23	Beef No. 1 unheated 1:10	24
16	Beef No. 1 unheated 1:20	24
23	Beef No. 1 heated 1:10	48
20	Beef No. 1 heated 1:20	48
20	Beef No. 2 unheated 1:10	48
21	Beef No. 2 unheated 1:20	36
24	Beef No. 2 heated 1:10	24
18	Beef No. 2 heated 1:20	24
16	Rabbit unheated 1:10	48
14	Rabbit unheated 1:20	24
18	Rabbit heated 1:10	24
18	Rabbit heated 1:20	24
17	Sterile salt solution (Cult. Con.)	24
16	Sterile sale solution (Cult. Con.)	24

20 M. L. D. (= 0.0004 c c culture diluted with salt solution up to 1 c c exposed to 1 c c of serum dilutions for one hour at 38 C.).
1 c c of 1:5 and 1:10 dilutions mixed with 1 c c of culture giving final dilutions of 1:10 and 1:20.
After the intraperitoneal injection of 1 c c of each mixture.

Additional experiments were made with what may be designated as a combined in vitro-vivo method by mixing in sterile test tubes a constant dose of culture with varying amounts of serum and after a period in the water bath at 38 C., injecting a portion of the contents of each tube into the peritoneal cavities of mice to determine the bactericidal activity of the serum. The results of one experiment of this nature consisting in exposing 20 minimal lethal doses of culture to 1 c c of 1:5 and 1:10 dilutions of unheated and heated normal beef and rabbit serums are shown in table 7; while the lives of several mice were prolonged about 24 hours beyond the culture controls, these experiments showed an absence or at best but a feeble bactericidal activity of normal beef serum even when used in amounts of 1 c c of undiluted serum.

THE BACTERICIDAL ACTIVITY OF NORMAL BEEF, RABBIT AND MOUSE SERUM FOR THE ANTHRAX BACILLUS IN VITRO

Numerous experiments were made for the purpose of eliciting evidence of possible bactericidal activity of normal beef serum for anthrax bacilli in vitro. Inasmuch as rabbits are highly immune and mice highly susceptible to anthrax, the serum or whole blood of these animals was usually included in our experiments for the double purpose of serving as controls and for a study of the nature of natural immunity to anthrax infection.

The technic and results of these experiments may be summarized as follows:

1. Fresh, sterile normal beef or rabbit serum unheated and heated twice at 56 C. were mixed in amounts of 5 c c with 0.5 c c of a thoroughly emulsified 24-hour broth culture of anthrax bacilli (practically no spores) and kept in a water bath at 38 C.; at intervals varying from 15 minutes to 4 hours 0.1 c c of each mixture was plated in 10 c c of agar at 42 C. and the plates counted after 48 hours' incubation. Culture controls were included in which sterile salt solution was used instead of serum. Neither beef nor rabbit serum showed any demonstrable bactericidal activity in these experiments.

2. Fresh sterile normal beef or rabbit serum unheated and heated were placed in sterile test tubes in constant amounts of 1 c c and treated with increasing amounts of thoroughly emulsified 24-hour broth cultures of anthrax bacilli varying from 0.01 to 0.1 c c; culture controls were included in which physiological salt solution replaced serum. After mixing and incubation in a water bath at 38 C. for 2 hours, 0.1 c c of each tube was plated with 10 c c of agar at 42 C. The general result of these tests with this technic was to show that sterile normal beef serum possesses some bactericidal activity for the anthrax bacillus while rabbit serum is without any demonstrable effect.

3. Best results were secured with the Heist-Lacy technic;[5] in this test six capillary tubes were employed with varying dilutions of culture (table 8). The bacilli exposed to serum or whole blood were those adhering to the interior of capillary tubes measuring about 1 mm. in diameter after the culture had been allowed to flow in for a distance of about 20 mm. followed by expulsion and filling with serum or blood to the same distance. Culture controls were included in which sterile broth was used instead of blood or serum. Each tubule was sealed and incubated for 24 hours when smears were made

[5] Jour. Immunology, 1918, 3, 3261.

and stained for anthrax bacilli. The results of one experiment are shown in table 8; plates prepared with 0.1 c c of each dilution of culture at the close of the experiment showed an uncountable number of anthrax colonies in the undiluted, 1:10, 1:100 and 1:1,000 dilutions of culture; the 1:10,000 dilution showed 9,400 colonies and the 1:100,000 dilution 1,850 colonies per c c.

The results of several experiments with this technic were unusually clear and decisive; either the smear showed very large numbers of bacilli, or none at all. Mouse blood invariably showed growths even with the 1:100,000 dilutions of 24-hour broth cultures, and these animals were highly susceptible to the culture employed; rabbit blood usually inhibited or killed the bacilli even with undiluted culture, and these animals were highly immune to intravenous injections of the culture. Sterile normal beef serum showed a high degree of bactericidal activity in this test and fresh unheated serum was usually, but not always, more germicidal than after heating twice at 56 C. for 30 minutes.

TABLE 8

RESULTS OF BACTERICIDAL TESTS WITH THE HEIST-LACY METHOD

Animal	Serum	Dilutions of Culture					
		Undiluted	1:10	1:100	1:1,000	1:10,000	1:100,000
Mouse	Fresh whole blood	+	+	+	+	+	+
Mouse	Fresh whole blood	+	+	+	+	+	+
Mouse	Fresh whole blood	+	+	+	+	+	+
Mouse	Fresh whole blood	+	+	+	+	+	+
Rabbit	Fresh whole blood	—	—	—	—	—	—
Rabbit	Fresh whole blood	—	—	—	—	—	—
Beef No. 1	Unheated serum	+	—	—	—	—	—
Beef No. 2	Unheated serum	—	—	—	—	—	—
Beef No. 3	Unheated serum	—	—	—	—	—	—
Beef No. 1	Heated serum	+	—	—	—	—	—
Beef No. 2	Heated serum	—	—	—	—	—	—
Beef No. 3	Heated serum	+	—	—	—	—	—

THE AGGLUTINATION OF THE ANTHRAX BACILLUS BY NORMAL BEEF, RABBIT AND MOUSE SERUM

Since it is generally accepted that agglutinins may aid the protective and curative properties of a serum by aiding phagocytosis and bacteriolysis, normal beef serum has been examined for agglutinins for anthrax bacilli. The majority of the serums were secured from specimens of blood collected in abattoirs and a few from cattle on a neighboring farm; nothing could be learned of the previous histories of these animals, and it is unknown whether or not any of them had had anthrax.

Rabbit and mouse serum was also tested to study the rôle of agglutinins in natural immunity to anthrax.

For the macroscopic agglutination tests antigens were prepared by these two methods:

(1) Flasks containing 200 c c of plain neutral broth were inoculated and grown at 42 C. for several days yielding rich and almost sporeless cultures;

at the end of this time 2 c c of liquor formaldehydi were added to each flask and the contents shaken in a mechanical shaker with beads until microscopic examination showed the thorough breaking up of the chains of bacilli. The antigens were allowed to stand for a day in a refrigerator and either centrifuged briefly or filtered through sterile cotton to remove clumps. The resulting antigen was of proper density, showed no spontaneous agglutination and stood up well for at least 36 hours at 55 C.

(2) Agar slant cultures were cultivated for 48 hours at 42 C. and washed off with 0.5% of liquor formaldehydi in salt solution, thoroughly shaken and briefly centrifuged or filtered through cotton after standing 24 hours. The resulting antigen was then diluted with liquor formaldehydi salt solution to the proper density. These antigens were somewhat inferior to those prepared by the first method and yielded lower agglutination titers.

TABLE 9

THE AGGLUTINATION OF B. ANTHRACIS BY NORMAL BEEF AND RABBIT SERUMS BY MACROSCOPIC TECHNIC

Serum	Highest Titers of the Serum of Different Animals													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Beef-unheated	1:96	1:160	1:144	1:192	1:192	1:224	1:120	1:18	1:48	1:56	1:32	1:24	1:40	1:48
Beef-heated	1:80	1:120	1:120	1:180	1:180	1:200	1:16	1:16	1:30	1:40	1:24	1:20	1:30	1:20
Rabbit-unheated	1:24	1:8	1:10	None	None	None	1:2	1:6	1:2	1:4	None	None	0	0
Rabbit-heated	1:6	1:2	None	None	None	None	None	1:4	None	None	None	None	0	0

TABLE 10

THE AGGLUTINATION OF B. ANTHRACIS BY NORMAL MOUSE AND RABBIT SERUMS BY MICROSCOPIC TECHNIC

Serum	Highest Titers of the Serum of Different Animals							
	1	2	3	4	5	6	7	8
Mouse—unheated	1:8	1:8	1:24	1:4	1:80	None	1:2	1:4
Rabbit—unheated	1:16	1:30	1:12	1:12	1:6	1:8	None	1:10

In the macroscopic tests each dilution of serum was used in a dose of 1 c c of anthrax antigen; the mixtures were kept at 55 C. for 16 to 18 hours and the results read. Salt solution controls were always included.

For the microscopic tests 24-hour broth cultures were thoroughly shaken in a mechanical shaker with beads and a loopful mixed on cover slides with a loopful of varying dilutions of serum. The results were read after one hour at room temperature. The controls occasionally showed some spontaneous agglutination requiring the frequent repetition of the tests.

The results of macroscopic agglutination tests with unheated and heated beef and rabbit serum are shown in table 9; all of these serums were tested within four days of the time of collection of the bloods.

As shown in table 9, normal beef serums contained agglutinin for our culture of anthrax but the amounts varied considerably in different animals as shown in a variation of titers ranging from 1:20 to 1:224; after heating once or twice at 56 C. for 30 minutes the titers were uniformly lower than those observed with fresh unheated serums.

While rabbits were highly immune to our culture of anthrax bacilli, their serum contained none or small amounts of agglutinin for this culture, the highest titer observed being 1:24.

The results of microscopic agglutination tests with unheated mouse and rabbit serums are shown in table 10; the highest titer observed with mouse serum was 1:80 while the majority were 1:8 or lower. With a macroscopic technic mouse serum yielded lower titers.

COMPLEMENT FIXATION WITH NORMAL BEEF, RABBIT AND MOUSE SERUMS AND ANTHRAX ANTIGENS

As a further study for anti-anthrax substances in normal beef serum, complement fixation tests were made; rabbit and mouse serums were included for purposes of comparison as representatives of animals possessing a high and low natural immunity to anthrax.

The antigens employed were those prepared after the methods described for the agglutination tests. Each antigen was used in a fresh state.

The tests were made with an antisheep hemolytic system, the antigen being titrated for anticomplementary activity just before the fixation tests and used in an amount equal to one-third the anticomplementary unit. All serums were freshly collected and tested unheated, and after heating at 56 C. for 30 minutes in graded doses varying from 0.01 to 0.2 c c. The usual serum antigen and hemolytic controls were included.

The primary incubation was conducted in a water bath at 38 C. for one hour; the secondary incubation was also in the water bath for about one hour depending on the hemolysis of the controls.

The results were entirely negative; 8 normal beef serums, 6 normal rabbit and 6 normal mouse serums yielded completely negative reactions with heated or unheated serum in the maximum dose of 0.2 c c. Occasionally a rabbit serum heated at 56 C. yielded a weakly positive reaction but all tests with active serum were negative and we ascribed these positive reactions with heated serums to the property of normal rabbit serums for fixing complement in a nonspecific manner with various lipoidal and bacterial antigens.

DISCUSSION

Normal beef serum may be said to possess some anti-anthrax properties by reason of its bactericidal properties in vitro demonstrated by the Heist-Lacy method, and to the presence of agglutinin for anthrax bacilli. The anti-anthrax properties of beef serum, however, are feeble, inasmuch as protection tests with mice in which the serum was injected intraperitoneally in doses varying from 10 to 100 c c per kilogram, and equivalent to 700 to 7,000 c c per 70 kilograms, or the weight of the average adult person, against one to five times the smallest amount of anthrax culture killing mice in a period of 48 hours, mainly yielded negative results. By reason of the virulence of the strain employed and the high susceptibility of the white

mouse, these tests may not have been sufficiently delicate to elicit minor degrees of protective power of the serums tested; rabbits, however, which Kraus employed in his experiments, possessed such a high degree of natural immunity to our culture of anthrax as to render them unsuitable and the results too favorable to the serum being tested.

A favorable opinion based on the results of the treatment of anthrax of persons with any serum may readily be based on error in view of the clinical course generally pursued by this infection. As anthrax is encountered among persons in Philadelphia, the mortality is very low providing the blood is sterile; recovery is prompt and without complications if the pustule is excised. In the experience of one of us (J. A. K.) anthrax bacteremia is generally fatal despite the administration of horse anti-anthrax serum, although recently a man suffering with a pustule on the face and whose blood showed the presence of anthrax bacilli, on repeated cultures, recovered without the use of serum. At best it would appear on the basis of these experiments that normal beef serum, as secured from animals under ordinary conditions, is but feebly protective or curative for anthrax, and while its administration as described by Penna and his associates may favorably influence the pustule, it is doubtful if the serum is sufficiently powerful to influence anthrax bacteremia.

In so far as our experiments bear on the nature of the natural immunity of the rabbit to anthrax and the high susceptibility of the white mouse, it would appear that the bactericidal activity of the whole blood is important in this relation. The blood of the adult rabbit is highly bactericidal as shown by the Heist-Lacy method, whereas that of the mouse is practically without effect on the anthrax bacillus. The presence or absence of agglutinins in the serum of these animals is not nearly so definite in relation to the question of natural immunity; generally, however, the serum of rabbits contains more agglutinin for anthrax bacilli than the serum of white mice, and the former possesses the higher degree of natural immunity to this particular infection.

SUMMARY AND CONCLUSIONS

Fresh sterile, normal beef serum is but feebly toxic for white mice by intraperitoneal injection; doses equivalent to 10 c c per 100 gm. of weight are well borne. Serums heated twice at 56 C. for 30 minutes are slightly less toxic.

Protection tests consisting in the intraperitoneal injection of mice with heated and unheated normal beef serum in doses ranging from 1 to 10 c c per 100 gm. generally failed to protect mice against 1 to 5 minimal lethal doses of culture.

By reason of the high susceptibility of white mice to anthrax these tests may not have been sufficiently delicate; the high degree of natural immunity to anthrax possessed by rabbits, however, renders them unsuitable for protection tests.

Unheated normal beef serum is bactericidal for the anthrax bacillus in vitro; heated serum is somewhat less bactericidal.

Rabbit blood is highly bactericidal for anthrax bacilli in vitro while mouse blood is without appreciable effect. The natural immunity of rabbits to anthrax is probably due in part to the bactericidal activity of their blood.

Normal beef serum contains variable amounts of agglutinin for anthrax bacilli, the titers ranging from 1:16 to 1:224; rabbit and mouse serum also contains small amounts of agglutinin, the former somewhat more than the latter. Heated serums of the three animals contains less agglutinin.

Normal beef, rabbit and mouse serums do not contain complement fixing substances for antigens of anthrax bacilli.

While normal beef serum contains some anti-anthrax substances they were found without demonstrable protective and curative value in experimental anthrax infections of mice and rabbits.